

REMARKS

In the outstanding Office Action, it was asserted that the pending claims are drawn to three inventions as set forth above.

In response thereto, claims 11-13 and 16-18 are provisionally elected with traverse, and claims 14-15, 19, and 20 are withdrawn from consideration pending the Examiner's consideration of the remarks below. In addition, new claims 21 and 22 have been added. Examination of the present application is respectfully requested.

Please note that prosecution before the U.S. Patent and Trademark Office in a national stage application based on a PCT application proceeds in the same manner as for a domestic application with the exception that unity of invention proceeds as under 37 CFR 1.475. See *MPEP* § 1893.03. Given that the present application is a national stage application of PCT application PCT/CN03/00003, the present claims must satisfy the unity of invention requirements as set forth in 37 CFR 1.475.

Applicant respectfully submits that the restriction requirement is in error, because the inventions recited in the claims are so linked as to form a single general inventive concept. According to 37 CFR 1.475, "the requirement of unity of invention shall be fulfilled only when there is a technical relationship among those inventions involving one or more of the same or corresponding special technical features." "Special technical features" means "those technical features that define a contribution which each of the claimed inventions, considered as a whole, makes over the prior art." Applicant respectfully submits that the contribution of the pending claims is a novel human Hepatoma-Derived Growth Factor 5 comprising the amino acid sequence of SEQ ID NO:2. Because the correlation between each amino acid and its corresponding codon is well known in the art, the nucleotide sequence of nucleotides 5-910 in SEQ ID NO:1 and the encoded amino acid sequence of SEQ ID NO:2 should be deemed as corresponding special technical features. Further, the special technical features shared in the pending claims render the claims patentable over the prior art.

Applicant further notes that, in four previously issued patents to the same inventor as the present case, a novel polypeptide and corresponding encoding nucleotide sequence were deemed as sharing special technical features. Please see the attached pages of the issued patents, identifying certain claims of each patent that emphasize this point.

Thus, it is respectfully submitted that the above restriction requirement is in error.
Reconsideration and withdrawal of the requirement is respectfully requested.

If a telephone conference would be helpful in resolving any remaining issues, please
contact the undersigned at 612-340-7862.

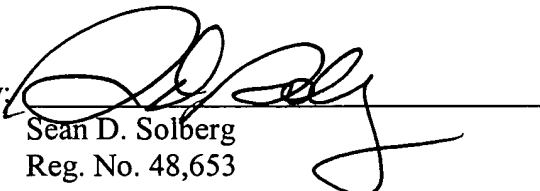
Respectfully submitted,

DORSEY & WHITNEY LLP
Customer Number 25763

Date:

October 26, 2006

By:


Sean D. Solberg
Reg. No. 48,653



US006743617B1

(12) **United States Patent**
Yu et al.

(10) Patent No.: **US 6,743,617 B1**
(45) Date of Patent: **Jun. 1, 2004**

(54) **HUMAN LYSOZYME GENE, ITS ENCODED POLYPEPTIDE AND THE METHOD FOR PREPARING THEM**

(75) Inventors: **Long Yu**, Institute of Genetics, Fudan University, Shanghai (CN); **Qiang Fu**, Shanghai (CN); **Yong Zhao**, Shanghai (CN); **Honglai Zhang**, Shanghai (CN); **Anding Bi**, Shanghai (CN)

(73) Assignee: **Long Yu**, Shanghai (CN)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/786,024**

(22) PCT Filed: **Aug. 30, 1999**

(86) PCT No.: **PCT/CN99/00132**

§ 371 (c)(1),
(2), (4) Date: **Jul. 5, 2001**

(87) PCT Pub. No.: **WO00/12723**

PCT Pub. Date: **Mar. 9, 2000**

(30) **Foreign Application Priority Data**

Aug. 31, 1998 (CN) 98111041 A

(51) Int. Cl.⁷ **C12N 4/36**

(52) U.S. Cl. **435/206; 435/183; 435/195; 435/252.3; 435/252.33; 435/255.1; 435/320.1; 536/23.2**

(58) Field of Search **435/183, 195, 435/206, 252.3, 252.33, 255.1, 320.1; 536/23.2**

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,643,758 A * 7/1997 Guan et al. 435/69.7

OTHER PUBLICATIONS

Hiller et al. Accession AA398583. Aug. 12, 1997.*

de Baetselier et al. Accession AAR05721. Aug. 16, 1990 (Alignment No. 1).*

de Baetselier et al. Accession AAR057521. Aug. 16, 1990 (Alignment No. 1).*

Jung et al. Accession V00428. Sep. 12, 1993 (Alignment No. 2).*

Online clipping from Entrez-PubMed, Messier W.S., "Episodic adaptive evolution of primate lysozymes", *Nature*, (6612), pp. 151-154, Jan. 1997.

Online clipping from Entrez-PubMed, Sippel et al., "Cloning of chicken lysozyme structural gene sequences synthesized in vitro", *Nucleic Acids Res.*, pp. 3275-3294, 1978.

Sava et al., "Lysozyme and Cancer: Role of Exogenous Lysozyme as Anticancer Agent (Review)", *Anticancer Research*, vol. 9, pp. 583-592, 1989.

"The Chemistry of Lysozyme and its use as a food preservative and a pharmaceutical", *Critical Review Food Science Nutrition*, vol. 26, issue 4, pp. 359-395, 1988.

Printout of various abstracts, including those herein.

Printout from NCBI Sequence Viewer regarding AC003687, Bireen et al., "*Homo sapiens* chromosome 17, clone HRPC29G21, complete sequence", 1998.

Printout from NCBI Sequence Viewer regarding M25446, Sippel et al., "Chicken lysozyme mRNA, partial cds", 1993. Printout from NCBI Sequence Viewer regarding P07232, Swanson et al., "Lysozyme C. Precursor (1, 4-Beta-N-Acetylmuramidase Cy)", Jul. 1998.

Printout from NCBI Sequence Viewer regarding P79847, Messier et al., "Lysozyme C Precursor (1, 4-Beta-N-Acetylmuramidase C)", *Nature* 385, pp. 151-154 Nov. 1997 (above).

* cited by examiner

Primary Examiner—Ponnathapu Achutamurthy

Assistant Examiner—Christian L. Fronda

(74) *Attorney, Agent, or Firm*—Baker Botts L.L.P.

(57) **ABSTRACT**

The invention relates to a novel member LYC3 of lysozyme gene family. The invention provides the cDNA sequence encoding for the novel lysozyme, the polypeptide encoded by the sequence, as well as the method for producing said novel human lysozyme utilizing recombinant technology. The invention also provides the use of the novel human lysozyme.

9 Claims, 2 Drawing Sheets

-continued

145

<210> SEQ ID NO 12
 <211> LENGTH: 147
 <212> TYPE: PRT
 <213> ORGANISM: Ring-necked pheasant

<400> SEQUENCE: 12

```

Met Arg Ser Leu Leu Ile Leu Val Leu Cys Phe Leu Pro Leu Ala Ala
1           5           10           15
Pro Gly Lys Val Tyr Gly Arg Cys Glu Leu Ala Ala Ala Met Lys Arg
          20           25           30
Met Gly Leu Asp Asn Tyr Arg Gly Tyr Ser Leu Gly Asn Trp Val Cys
          35           40           45
Ala Ala Lys Phe Glu Ser Asn Phe Asn Thr Gly Ala Thr Asn Arg Asn
          50           55           60
Thr Asp Gly Ser Thr Asp Tyr Gly Ile Leu Gln Ile Asn Ser Arg Trp
          65           70           75           80
Trp Cys Asn Asp Gly Arg Thr Pro Gly Ser Lys Asn Leu Cys His Ile
          85           90           95
Pro Cys Ser Ala Leu Leu Ser Ser Asp Ile Thr Ala Ser Val Asn Cys
          100          105          110
Ala Lys Lys Ile Val Ser Asp Gly Asn Gly Met Asn Ala Trp Val Ala
          115          120          125
Trp Arg Lys His Cys Lys Gly Thr Asp Val Asn Val Trp Ile Arg Gly
          130          135          140
Cys Arg Leu
145

```

What is claimed is:

1. An isolated DNA molecule comprising a nucleotide sequence encoding a protein comprising the amino acid sequence of SEQ ID NO:4, wherein said protein has lysozyme activity.
2. The DNA molecule of claim 1 wherein said nucleotide sequence comprises the nucleotide sequence of nucleotides 81-521 of SEQ ID NO:3.
3. A vector containing the DNA sequence of claim 1.
4. A host cell transformed by the vector of claim 3.
5. The host cell of claim 4 which is *E. coli*.
6. The host cell of claim 4 which is a eukaryotic cell.
7. A method for producing an LYC3 protein having lysozyme-activity comprising:
 - (a) introducing an expression vector for production of LYC3 protein, said vector comprising a nucleotide sequence encoding a protein having the amino acid sequence of SEQ ID NO:4 or of amino acids 19-146 of SEQ ID NO:4, wherein said nucleotide sequence is operably linked to at least one expression control sequence, into a host cell, thereby forming a recombinant host cell;
 - (b) culturing the recombinant host cell of (a) under conditions suitable for expression of the DNA molecule encoding the protein, such that LYC3 protein is produced; and
 - (c) isolating the LYC3 protein so produced,
8. An isolated LYC3 protein having lysozyme activity comprising a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:4 and amino acids 19-146 of SEQ ID NO:4.
9. An isolated DNA molecule having a nucleotide sequence encoding a lysozyme consisting of the amino acid sequence of amino acids 19-146 of SEQ ID NO:4, wherein said lysozyme has lysozyme activity.

* * * * *



US006436688B1

(12) **United States Patent**
Yu et al.

(10) Patent No.: **US 6,436,688 B1**
(45) Date of Patent: **Aug. 20, 2002**

(54) **HUMAN LYSOZYME GENE, ITS ENCODING POLYPEPTIDE AND THE METHOD PREPARING FOR THEM**

(75) Inventors: **Long Yu; Qiang Fu; Yong Zhao; Honglai Zhang; Anding Bi**, all of Shanghai (CN)

(73) Assignee: **Institute of Genetics, Shanghai (CN)**

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/786,023**

(22) PCT Filed: **Aug. 30, 1999**

(86) PCT No.: **PCT/CN99/00131**

§ 371 (c)(1),

(2), (4) Date: **Jun. 21, 2001**

(87) PCT Pub. No.: **WO00/12722**

PCT Pub. Date: **Mar. 9, 2000**

(30) **Foreign Application Priority Data**

Aug. 31, 1998 (CN) 98111039 A

(51) Int. Cl.⁷ **C12N 9/36; C07H 21/04**

(52) U.S. Cl. **435/206; 435/325; 435/252.33; 435/320.1; 536/23.2; 536/23.5**

(58) Field of Search **435/206, 320.1, 435/252.33, 325; 536/23.2, 23.5**

(56) **References Cited**

PUBLICATIONS

Hillier et al. Data Base: EST, Accession No.: AA416679, GI: 2077631 (Nov. 9, 1997).*

NCI-CGAP, Data Base: EST, Accession No.: AI066607, GI: 3367309 (Aug. 27, 1998).*

Online clipping from Entrez-PubMed, Dautigny et al., "cDNA and amino acid sequences of rainbow trout (*Oncorhynchus mykiss*) lysozymes and their implications for the evolution of lysozyme and lactalbumin", Dautigny et al. J. Mol. Evol., vol. 32, issue 2, pp. 187-198, Feb. 1991.

Yajima et al. vol. 26, Issue 4 (1988). pp. 373-395. CRC Clinical Reviews in Food Science and Nutrition.

Mol Biol Evol Nov. 1994: 11(6) 921-8 Molecular adaptation of a leaf-eating bird: Stomach lysozyme of the hoatzin by Kornegay, Jr. et al.

J Biochem (Tokyo) Feb. 1982: 91 (2) 571-87 Chemical and immunological properties and amino acid sequences of three lysozymes from Peking—duck egg white; by Kondo et al.

Online clipping from Entrez-PubMed, Weisman et al., "Evolutionary shift in the site of cleavage of prelysosome", J. Biol. Chem., vol. 261, issue 5, pp. 2309-2313, 1986.

J Mol. Evol. Nov. 1991: 33(5) 418-25 "Stomach lysozyme gene of the langur monkey: tests for convergence and positive selection" by Swanson et al.

Nature 1997; Jan. 9: 385(6612) 151-4 "Episodic adaptive evolution of primate lysozymes" by Messier.

* cited by examiner

Primary Examiner—Nashaat T. Nashed

(74) Attorney, Agent, or Firm—Baker Botts LLP

(57) **ABSTRACT**

The invention relates to a novel member LYC2 of lysozyme gene family. The invention provides the cDNA sequence encoding for the novel lysozyme, the polypeptide encoded by the sequence, as well as the method for producing said novel human lysozyme utilizing recombinant technology. The invention also provides the use of the novel human lysozyme.

12 Claims, 2 Drawing Sheets

-continued

Cys Arg Leu
145

<210> SEQ ID NO 12
<211> LENGTH: 146
<212> TYPE: PRT
<213> ORGANISM: Red Guenon

<400> SEQUENCE: 12

Met Lys Ala Val Ile Ile Leu Gly Leu Val Leu Leu Ser Val Thr Val
1 5 10 15
Gln Gly Lys Ile Phe Glu Arg Cys Glu Leu Ala Arg Thr Leu Lys Arg
20 25 30
Leu Gly Leu Asp Gly Tyr Arg Gly Ile Ser Leu Ala Asn Trp Val Cys
35 40 45
Leu Ala Lys Trp Glu Ser Gly Tyr Asn Thr Gln Ala Thr Asn Tyr Asn
50 55 60
Pro Gly Asp Gln Ser Thr Asp Tyr Gly Ile Phe Gln Ile Asn Ser His
65 70 75 80
Tyr Trp Cys Asn Asn Gly Lys Thr Pro Gly Ala Val Asn Ala Cys His
85 90 95
Cys Asn Ala Leu Leu Gln Asp Asn Ile Ala Asp Ala Val Thr Cys Ala
100 105 110
Lys Arg Val Val Arg Asp Pro Gln Gly Ile Arg Ala Trp Val Ala Trp
115 120 125
Arg Asn His Cys Gln Asn Arg Asp Val Ser Gln Tyr Val Gln Gly Cys
130 135 140
Gly Val
145

What is claimed is:

1. An isolated DNA molecule having a nucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO:4 or amino acids 20-148 of SEQ ID NO:4.
2. The DNA molecule of claim 1 wherein said nucleotide sequence encodes a polypeptide having the amino acid sequence of amino acids 20-148 of SEQ ID NO: 4.
3. The DNA molecule of claim 1 wherein said nucleotide sequence has the nucleotide sequence of nucleotides 106-552 of SEQ ID NO: 3.
4. An isolated LYC2 polypeptide having the amino acid sequence of SEQ ID NO: 4 or of amino acids 20-148 of SEQ ID NO: 4.
5. The polypeptide of claim 4 wherein said polypeptide has the amino acid sequence of amino acids 20-148 of SEQ ID NO: 4.
6. A vector containing the DNA sequence of claim 1.
7. A host cell transformed by the vector of claim 6.
8. The host cell of claim 7 which is *E.coli*.
9. The host cell of claim 7 which is a eukaryotic cell.

10. A method for producing a LYC2 protein which comprises:

- (a) introducing an expression vector for producing a LYC2 protein, said vector comprising a nucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO:4 or of amino acids 20-148 of SEQ ID NO:4, wherein said nucleotide sequence is operably linked to at least one expression control sequence, into a host cell, thereby forming a recombinant host cell;
 - (b) culturing the recombinant host cell of (a) under conditions suitable for expression of the DNA molecule encoding the polypeptide, such that LYC2 protein is produced; and
 - (c) isolating the LYC2 protein so produced.
11. The method of claim 10 wherein said nucleotide sequence comprises nucleotides 106-552 of SEQ ID NO: 3.
 12. An isolated nucleotide molecule which is the antisense sequence of the DNA molecule of claim 1.

* * * * *



US006943244B2

(12) **United States Patent**
Yu et al.

(10) Patent No.: **US 6,943,244 B2**

(45) Date of Patent: **Sep. 13, 2005**

(54) **HUMAN G-TYPE LYSOZYME, THE
ENCODING SEQUENCE, PREPARING
METHOD AND THE USES THEREOF**

(75) Inventors: **Long Yu**, Institute of Genetics, Fudan
University, 220 Handan Road, Shanghai
(CN), 200433; **Yong Zhao**, Shanghai
(CN); **Peirong Hu**, Shanghai (CN);
Lisha Tang, Shanghai (CN); **Shouyuan
Zhao**, Shanghai (CN)

(73) Assignee: **Long Yu**, Shanghai (CN)

(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 169 days.

(21) Appl. No.: **10/469,602**

(22) PCT Filed: **Jul. 17, 2001**

(86) PCT No.: **PCT/CN01/01176**

§ 371 (c)(1),

(2), (4) Date: **Sep. 2, 2003**

(87) PCT Pub. No.: **WO02/070715**

PCT Pub. Date: **Sep. 12, 2002**

(65) **Prior Publication Data**

US 2004/0072292 A1 Apr. 15, 2004

(30) **Foreign Application Priority Data**

Mar. 2, 2001 (CN) 1105523 A

(51) Int. Cl.⁷ C12N 15/56; C12N 15/70;
C12N 15/79; C12N 9/36; C12Q 1/68

(52) U.S. Cl. **536/23.2**; 435/69.1; 435/6;
435/206; 435/252.3; 435/252.33; 435/320.1

(58) Field of Search 536/23.2; 435/6;
435/69.1, 206, 252.3, 252.33, 320.1

(56) **References Cited**

U.S. PATENT DOCUMENTS

6,083,700 A	7/2000	Lal et al.	435/6
6,268,164 B1 *	7/2001	Lal et al.	435/18
6,528,297 B1	3/2003	Yu et al.	435/207
6,660,485 B2 *	12/2003	Lal et al.	435/7.1
2002/0111302 A1 *	8/2002	Tang et al.	514/12
2004/0101930 A1 *	5/2004	Jackson et al.	435/69.1

FOREIGN PATENT DOCUMENTS

WO	WO 2001/98353 A1 *	12/2001
WO	WO 2002/22802 A2 *	3/2002
WO	WO 2002/44340 A2 *	6/2002

* cited by examiner

Primary Examiner—Ponnathapura Achutamurthy

Assistant Examiner—William W. Moore

(74) *Attorney, Agent, or Firm*—Merchant & Gould P.C.

(57) **ABSTRACT**

The present invention provided a novel human goose-type lysozyme (LYG2) and the polynucleotide encoding the LYG2. The invention also provided the corresponding expression vectors, host cells, antibodies, agonists and antagonists. The invention also provided the method for diagnosis, treatment and prevention of the diseases relative to LYG2 expression.

9 Claims, No Drawings

-continued

100	105	110	
gac ggc tgg gac cac agg gga ctt aaa ttt ggc ttg atg cag ctt gat			500
Asp Gly Trp Asp His Arg Gly Leu Lys Phe Gly Leu Met Gln Leu Asp			
115	120	125	
aaa caa acg tac cac cct gtc ggt gcc tgg gat agc aaa gag cac ctt			548
Lys Gln Thr Tyr His Pro Val Gly Ala Trp Asp Ser Lys Glu His Leu			
130	135	140	145
tca cag gct act ggg att cta aca gag aga att aag gca atc cag aaa			596
Ser Gln Ala Thr Gly Ile Leu Thr Glu Arg Ile Lys Ala Ile Gln Lys			
150	155	160	
aaa ttc ccc acg tgg agt gtt gct cag cac ctc aaa ggt agg ctg tat			644
Lys Phe Pro Thr Trp Ser Val Ala Gln His Leu Lys Gly Arg Leu Tyr			
165	170	175	
tct gag tac ttt gtt taa atgagcaatg aatgagacca ctgaagacca			692
Ser Glu Tyr Phe Val			
180			
gtgtgacccg agactccctg ggagcatttc cacggggcca gcagtggccc tgggaggagc			752
tgcttagagg ctgcatttgc attcctgaa ccaactgagtt actttgagag gtcctccatc			812
ctcaacctcc atttcctctt ctgcagaatg ttgg			846
<210> SEQ ID NO 3 <211> LENGTH: 23 <212> TYPE: DNA <213> ORGANISM: Artificial <220> FEATURE: <221> NAME/KEY: misc_feature <222> LOCATION: (1)..(23) <223> OTHER INFORMATION: synthetic oligonucleotide			
<400> SEQUENCE: 3			
gtaaggttgc aaacaaggtc ctg			23
<210> SEQ ID NO 4 <211> LENGTH: 23 <212> TYPE: DNA <213> ORGANISM: Artificial <220> FEATURE: <221> NAME/KEY: misc_feature <222> LOCATION: (1)..(23) <223> OTHER INFORMATION: synthetic oligonucleotide			
<400> SEQUENCE: 4			
tcacactggt cttcagtggt ctc			23

What is claimed is:

1. An isolated and purified polynucleotide encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 1.

2. An isolated and purified polynucleotide comprising the contiguous nucleotide sequence of nucleotides 1-846 or of nucleotides 114-662 of SEQ ID NO: 2.

3. An isolated and purified polynucleotide which is complementary to the polynucleotide of claim 1.

4. An expression vector comprising the polynucleotide of claim 1.

5. A host cell comprising the expression vector of claim 4.

6. A method for producing a polypeptide, the method comprising the steps of:

(a) culturing the host cell of claim 5 under conditions suitable for the expression of the polypeptide; and

(b) recovering the polypeptide from the host cell culture.

7. A method for detecting a polynucleotide encoding a polypeptide having lysozyme activity in a biological sample containing nucleic acids, the method comprising the steps of:

(a) hybridizing the polynucleotide of claim 3 to at least one of the nucleic acids of the biological sample under stringent conditions with a wash step in conditions of 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS at a temperature of at least 68° C., thereby forming a hybridization complex; and

(b) detecting the hybridization complex, wherein the presence of the hybridization complex correlates with the presence of a polynucleotide encoding the polypeptide in the biological sample;

whereby the polynucleotide encoding a polypeptide having lysozyme activity is detected.

37

8. A method of screening a library of molecules or compounds to identify at least one molecule or compound which specifically binds a polynucleotide encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 1 under stringent conditions, the method comprising the steps of:

- (a) combining an isolated and purified polynucleotide encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 1 with a library of molecules or compounds under conditions to allow specific bind-

38

ing under stringent conditions with a wash step in conditions of 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS at a temperature of at least 68° C.; and

- (b) detecting specific binding;

thereby identifying a molecule or compound which specifically binds the polynucleotide.

9. An isolated and purified polypeptide comprising the amino acid sequence of SEQ ID NO:1.

* * * * *





US006660512B1

(12) **United States Patent**
Yu et al.

(10) Patent No.: **US 6,660,512 B1**
(45) Date of Patent: **Dec. 9, 2003**

(54) **HUMAN LYSOZYME GENE, IT'S ENCODED POLYPEPTIDE AND THE METHOD OF PREPARING THEM**

(75) Inventors: **Long Yu**, Shanghai (CN); **Qiang Fu**, Shanghai (CN); **Yong Zhao**, Shanghai (CN); **Honglai Zhang**, Shanghai (CN); **Anding Bi**, Shanghai (CN)

(73) Assignee: **Yu Long**, Shanghai (CN)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/786,025**

(22) PCT Filed: **Aug. 30, 1999**

(86) PCT No.: **PCT/CN99/00133**

§ 371 (c)(1),

(2), (4) Date: **Jun. 8, 2001**

(87) PCT Pub. No.: **WO00/12717**

PCT Pub. Date: **Mar. 9, 2000**

(30) **Foreign Application Priority Data**

Aug. 31, 1998 (CN) 98111044 A

(51) Int. Cl.⁷ **C12N 9/36; C12N 15/56**

(52) U.S. Cl. **435/206; 435/200; 435/320.1; 435/252.3; 435/252.33; 435/69.1; 435/71.1; 435/325; 536/23.2; 536/23.5**

(58) Field of Search **435/206, 200, 435/320.1, 252.3, 252.33, 69.1, 71.1, 325; 536/23.2, 23.5**

(56) **References Cited**

FOREIGN PATENT DOCUMENTS

WO WO99/09155 * 2/1999 C12N/15/11

OTHER PUBLICATIONS

Brewer LA, Duan R et al, Human secreted protein, cDNA fragment containing gene 36. Feb. 1999 Alignment SEQ ID No.: 3.*

Brewer LA, Duan R et al, Human secreted protein fragment encoded from gene 36. Feb. 1999 Alignment with SEQ ID No.: 4.*

Strausberg R. NCI, Cancer Genome Anatomy Project (CGAP), Tumor Gene Index Dec. 1998, Alignment with SEQ ID No.: 3.*

NCI-GAP National Cancer Inst, Cancer Genome Anatomy Project <http://www.ncbi.nlm.nih.gov/ncicgap> Dec. 31, 1998 EMBL Acc# AA812577 Alignment with SEQ ID No.: 4.* Agric Biol Chem 1991, Jul.;55(7):1707-13 The amino acid sequence of reeves' pheasant (syrmaticus reevesii) Lysozyme.

Acta Otolaryngol (Stockh) Nov. 1997; 117(6):851-855. "The effects of oxymetazoline on Lysozyme secretion from the human nasal mucosa".

Yajima et al. vol. 26, Issue 4 (1988). pp. 373-395. CRC Clinical Reviews in Food Science and Nutrition.

Anticancer Research 9: 583-592 (1989) Lysozyme and Cancer: Role of Exogenous Lysozyme as Anticancer Agent (Review) by Sava et al.

Lubbert M. Henschler R, Kreutz M, Andreesen R. Mertelsmann R, Herrmann F, "The human lysozyme gene undergoes stepwise demethylation during phagocyte maturation" *Leukmia* Jul. 1997; 11(7):990 (Abstract).

Mendeluk GR, Blanco AM, Bregni C, "Viscosity of human seminal fluid: role of lysomzyme" *Arch Androl* Jan.-Feb. 1997;38(1):7 (Abstract).

Bentley GA, "The crystal structures of complexes formed between lysozyme and antibody fragments" *EXS* 1996;75:301 (Abstract).

Dupont C, Kluepfel D, Morosoli R, "Evidence for lysozyme-type mechanism of hydrolysis in xylanases" *EXS* 1996;75:411 (Abstract).

Fischer B, "Folding of lysozyme" *EXS* 1996;75:143 (Abstract).

Holtje JV, "Lysozyme substrates" *EXS* 1996; 75:105 (Abstract).

Imoto T, "Engineering of lysozyme" *EXS* 1996;75:163 (Abstract).

Irwin DM, Yu M, Wen Y, "Isolation and characterization of vertebrate lysozyme genes" *EXS* 1996;75:225 (Abstract).

Karplus M, Post CB, "Simulations of lysozyme: internal motions and the reaction mechanism" *EXS* 1996;75:111 (Abstract).

Prager EM, "Adaptive evolution of lysozyme: changes in amino acid sequence, regulation of expression and gene number" *EXS* 1996;75:323 (Abstract).

Short ML, Nickel J, Schmitz A, Renkawitz R, "Lysozyme gene expression and regulation" *EXS* 1996;75:243 (Abstract).

Wang YB, "Antibacterial mechanisms of lysozyme on *Streptococcus mutans*" *Zhonghua Ya Yi Xue Hui Za Zhi* Sep. 1990;9(3):87 (Abstract).

Chung LP, Keshav S, Gordon S, "Cloning the human lysozyme cDNA: inverted Alu repeat in the mRNA and in situ hybridization for macrophages and Paneth cells" *Proc Natl Acad Sci USA* Sep. 1988;85(17):6227 (Abstract).

Castanon MJ, Spevak W, Adolf GR, Chlebowicz-Sledziowska E, Sledziwski A, "Cloning of human lysozyme gene and expression in the yeast *Saccharomyces cerevisiae*" *Gene* Jun. 30, 1998;66(2):223 (Abstract).

Saedi MS, Garvey KJ, Ito J, "Cloning and purification of a unique lysozyme produced by *Bacillus* phage phi 29" *Proc Natl Acad Sci USA* Feb. 1987;84(4):955 (Abstract).

(List continued on next page.)

Primary Examiner—Rebecca E. Prouty

Assistant Examiner—Sheridan L Swope

(74) Attorney, Agent, or Firm—Baker Botts L.L.P.

(57) **ABSTRACT**

The invention relates to LYC4, a novel member of the lysozyme gene family. The invention provides the cDNA sequence encoding the novel lysozyme, the polypeptide encoded by the sequence, as well as methods for producing said novel human lysozyme utilizing recombinant technology. The invention also provides the use of the novel human lysozyme.

12 Claims, 1 Drawing Sheet

-continued

Ala Lys Phe Glu Ser Asn Phe Asn Thr Gly Ala Thr Asn Arg Asn Thr
 35 40 45

Asp Gly Ser Thr Asp Tyr Gly Ile Leu Gln Ile Asn Ser Arg Trp Trp
 50 55 60

Cys Asn Asp Gly Arg Thr Pro Gly Ser Lys Asn Leu Cys His Ile Pro
 65 70 75 80

Cys Ser Ala Leu Leu Ser Ser Asp Ile Thr Ala Ser Val Asn Cys Ala
 85 90 95

Lys Lys Ile Val Ser Asp Gly Asp Gly Met Asn Ala Trp Val Ala Trp
 100 105 110

Arg Lys His Cys Lys Gly Thr Asp Val Asn Val Trp Ile Arg Gly Cys
 115 120 125

Arg Leu
 130

What is claimed is:

1. An isolated DNA molecule having a nucleotide sequence encoding a polypeptide having the amino acid sequence of amino acids 20-146 of SEQ ID NO:4. 25

2. The DNA molecule of claim 1 wherein said nucleotide sequence encodes a polypeptide having the amino acid sequence of SEQ ID NO:4.

3. The DNA molecule of claim 1 wherein said nucleotide sequence has the nucleotide sequence of nucleotides 179-619 of SEQ ID NO:3. 30

4. An isolated lysozyme polypeptide having the amino acid sequence of amino acids 20-146 of SEQ ID NO:4.

5. The polypeptide of claim 4 wherein said polypeptide has the amino acid sequence of SEQ ID NO:4. 35

6. A vector containing the DNA sequence of claim 1.

7. A host cell transformed by the vector of claim 6.

8. The host cell of claim 7 which is *E.coli*.

9. The host cell of claim 7 which is a eukaryotic cell. 40

10. A method for producing a LYC4 protein which comprises:

(a) introducing an expression vector for producing a LYC4 protein, said vector comprising a nucleotide sequence encoding a polypeptide having the amino acid sequence of amino acids 20-146 of SEQ ID NO:4, wherein said nucleotide sequence is operably linked to at least one expression control sequence, into a host cell, thereby forming a recombinant host cell;

(b) culturing the recombinant host cell of (a) under conditions suitable for expression of the DNA molecule encoding the polypeptide, such that LYC4 protein is produced; and

(c) isolating the LYC4 protein so produced.

11. The method of claim 10 wherein said nucleotide sequence comprises nucleotides 179-619 of SEQ ID NO:3.

12. An isolated nucleotide molecule which is the antisense sequence of the DNA molecule of claim 1.

* * * * *